UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH ADMINISTRATION
Bureau of Animal Industry

BRUCELLA ABORTUS STRAIN 19 VACCINATION

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Research Council by C. K. Mingle)

Although a great deal has been published on Strain 19 vaccine during the past several years, it may be well for purposes of discussion by this group to review some of the experimental background upon which the prophylactic value of the culture was determined.

Origin of Strain 19

Strain 19 was first isolated in 1923 by Dr. J. M. Buck of the Bureau of Animal Industry's Animal Disease Station, Beltsville, Maryland, from the milk of a cow in an experimental herd maintained by the Bureau of Dairy Industry. This cow was a pure-bred Jersey, identified in the American Jersey Cattle Club as "Victor's Lady Matilda." She was born on May 3, 1915, in Grove, Pennsylvania, and was eventually purchased by the Bureau of Dairy Industry in 1921 for use in a family-crossing experiment. Aside from her scientific contribution to brucellosis research, Matilda was no ordinary cow. She made two production records, one of 7,220 pounds of milk, 350 pounds butter fat, at 2 years 9 months of age; and the other, 10,861 pounds of milk, 584 pounds butter fat at 5 years 4 months of age. After having her third normal calf, Matilda became sterile. There is no record of her ever having aborted, but she did become a brucellosis reactor in 1923. In June 1923, Dr. Buck recovered a culture of Brucella abortus from the milk of Matilda which was later to become known as Strain 19. At the time of isolation, the culture was fully virulent, and it was only after it had been held on artificial media for about two years that the virulence became reduced to its present low grade.

The most unusual quality of Strain 19 has been the stability of the reduced virulence that has characterized it for so many years.

Immunology

In vaccination studies conducted by Dr. Buck between 1925 and 1929, he observed that one of his test cultures (Strain 19) was capable of producing a serviceable immunity in calves to brucellosis without causing the disease. This marked the beginning of a series of comprehensive vaccination experiments with Strain 19 that finally led to the official recognition by the Bureau in December 1940 of vaccination of calves as an aid in cooperative brucellosis control work.

The nature of the repeated experiments conducted under rigidly controlled conditions was essentially the vaccination of calves with Strain 19 and their subsequent eye exposure during the middle third of gestation, with an equal number of controls, to a pre-determined amount of virulent Brucella abortus. In the interest of brevity, details of individual tests will be omitted. Briefly, out of the 70 animals vaccinated during calfhood in the early experiments, 84.3 percent were fully protected against the same degree of artificial exposure that established active Brucella infection in 78.1 percent of the unvaccinated controls. Further work conducted at a later date, in which comparable data were available confirms, to a large extent, the above figures.

In order to determine the effectiveness of Strain 19 vaccination under natural conditions, extensive field trials were carried out over a five year period extending from 1936 to 1941. For this study a total of 260 herds having a minimum of 15 percent infection were selected. These herds

were located in 24 different States, and although no controls were provided, the owners were encouraged to hold infected animals in the herds to provide as much exposure as possible for the vaccinates. In all, there were 19,629 animals vaccinated during calfhood. From these there were 14,280 calvings, involving as many as five pregnancies, 13,804, or 96.7 percent of which were normal. Of the 476 animals that aborted (3.3 percent of total pregnancies), 298 or 62.6 percent were negative to the agglutination test at the time of calving. On the basis of the blood agglutination test alone, only 178 abortions, or 1.2 percent of the total calvings were attributed to brucellosis.

These results helped to substantiate the findings from controlled experiments and underlined the value of Strain 19 vaccination of calves in producing a serviceable degree of resistance to subsequent Brucella abortus exposure. It was on the basis of these conclusions that the aforementioned Bureau recognition of vaccination as an official part of the brucellosis control program was proposed.

Stability of Strain 19

Some of the hardest criticism to answer regarding Strain 19 vaccination has been the expressed fear that the reduced virulence of the culture may become enhanced in the animal's body and serve as a means of spreading active infection. In order to provide additional data on this subject, deliberate attempts were made to increase the virulence of Strain 19 by subjecting it to every condition known that might favor reversion. Included in these studies was a series of passages through susceptible first calf heifers. By mass intravencus exposures of approximately 90 billion Strain 19 organisms, it was possible to cause pregnant cattle to abort. Recoveries made from original abortions were, in turn, used to similarly expose each succeeding animal until a total of 6 passages of the culture had been made. It was

significant to find that the final recovery made from the sixth aborting principal was indistinguishable from the original stock strain as determined by a careful comparison of morphological, cultural and virulent qualities.

These results, together with similar observations made in connection with both controlled and field vaccination studies are strong support for the belief that Strain 19 will not assume dangerous pathogenic properties under conditions in which its use is recommended.

Adult Vaccination

The recognized disadvantages of persistent blood agglutination reactions that are associated with Strain 19 vaccination of mature animals places this procedure in a position where its intelligent use is greatly restricted. Although there has been very little controlled research carried out on adult vaccination, there is reason to believe that it will induce an equal, if not greater and more lasting protection in healthy animals than is true for vaccination of calves. Unfortunately, the blood reactions elicited by Strain 19 cannot be distinguished from those due to virulent infection. Such being the case, all control of the extent and possible spread of virulent infection in a herd, which would otherwise be provided by the agglutination test, is lost for an indefinite period in adult vaccinated animals.

In view of the fact that a great many adult animals are being vaccinated outside of the official program, it is believed that it would be better to permit its restricted use under official supervision, than to encourage promiscuous, uncontrolled adult vaccination by prohibiting it entirely. In any event, whole-herd vaccination must be considered an emergency measure, to be adopted only under conditions where suitable precautions can be taken to minimize all possible dangers connected with its use.

Preparation of Strain 19 Vaccine

In order to insure maximum benefits from Strain 19 vaccination, it has been necessary to carry out considerable research on the production and distribution of the vaccine. From these studies, standardized methods for vaccine production have been set up. Outlines of these recommendations are supplied to all commercial producers as a guide for them to follow. This helps to maintain a more uniform product than would be possible if a wide variety of methods were employed. Experience has shown that there are a number of vulnerable steps in the production of vaccine that must be carefully handled to avoid the occurrence of undesirable changes in the finished product. Dissociation of Strain 19, for example, takes place very rapidly during certain stages of vaccine production unless appropriate precautions are exercised.

The attached directions for the production of Brucella abortus Strain 19 vaccine is an exact copy of those provided commercial producers and can be discussed at whatever length the Subcommittee desires.

It is required that the product be distributed in single dose vials, each containing 6 cc. of vaccine, and that it carry suitable labels showing the date at which the allowed three months expiration period ends. This expiration period is figured from the actual date on which the organisms are harvested. The recommended dose is 5 cc. administered subcutaneously. While no exact injection site is specified, vaccinations are usually performed either directly anterior or posterior to the shoulder. The intradermal administration of Strain 19 vaccine is not recognized at the present time as an official method.

Bureau Control of Strain 19 Vaccine Production

Through the Division of Virus Serum Control, close supervision is maintained on all Strain 19 vaccine produced on a commercial scale. Eight samples are picked up by field inspectors from each serial lot of vaccine and forwarded to the Animal Disease Station Laboratory at Beltsville, Maryland, for testing. The factors determined by this examination are:

1. Purity.
2. Viability - a minimum of 10 billion viable Brucella organisms per cubic centimeter is required.
3. Dissociation - not more than 15 percent dissociated forms are permitted.
4. Hydrogen-ion concentration of the suspension - a pH of 6.3 is preferred, with tolerable limits of 5.9 to 6.3.

It is only after these tests have been satisfactorily met that the serial lot in question is released for marketing. In addition to tests made at the time of production, samples may be obtained by Bureau inspectors from field depots any time during the expiration period. If, as a result of such check tests, the viability of a given serial is found to be lower than 5 billion Brucella abortus organisms per cubic centimeter, all the remaining product bearing the same serial number is immediately taken off the market. This precaution helps to insure proper handling of the vaccine in the field.

At the present time there are 18 licensed establishments producing liquid Strain 19 vaccine. The attached Table I summarizes the amount of commercial vaccine produced each year beginning in 1939, and records the condemnations made by the Bureau for the same periods. It will be noted that the batch

condemnations have been reduced from a high of 21.6 percent in 1939 to around 7 percent in 1946, and that the quantity of vaccine produced in 1946 was higher than in any previous year. The fact that something like 3-1/2 million doses were manufactured by commercial houses in 1946 with less than 1 million calves being vaccinated on an official basis during the same period, emphasizes the probable extent to which uncontrolled vaccination is being practiced.

Bureau of Animal Industry Vaccine Production

The Bureau continues to make and distribute approximately 200,000 cc. of Strain 19 vaccine each year. This is distributed throughout the various States in as equitable a manner as possible. The primary purpose behind the Bureau's preparation of vaccine is that it serves as a valuable aid in supervising commercial production methods, and by distributing the vaccine in a large number of areas, it provides control groups of animals against which the performance of commercial vaccines can be compared.

With the limited facilities and personnel available at the Animal Disease Station, and because of the heavy volume of commercial testing that is required, it is doubtful if production of Strain 19 vaccine will ever be increased over the present level. As a matter of fact, it is entirely possible that the current volume may have to be reduced in order to carry out the additional supervision required by increasing commercial production.

Desiccated Vaccine

During the past few years the possibility of adapting the process of drying from the frozen state (lyophilization) to Strain 19 vaccine production has received increasing attention. While a great many commercial laboratories are keenly interested in the procedure, only one has so far marketed a dry

product. One of the principle reasons for this has been the extreme difficulty encountered in standardizing the methods employed so that unusually heavy losses in viability do not occur during the actual drying. If these technical details can be worked out, there is reason to believe that lyophilization will eventually provide the answer to the present problem of handling the highly sensitive liquid suspension. There is no evidence at this time that the antigenic qualities of viable Strain 19 cells are changed by lyophilization.

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TABLE I. TESTS ON COMMERCIAL BRUCELLA ABORTUS STRAIN 19 VACCINE

(1939-1946)

	BATCHES			CUBIC CENTIMETERS		
Year	Number Tested	Number Condemned	Percent Condemned	Number Tested	Number Condemned	Percent Condemned
1939	1,092	237	21.6	---	---	---
1940	1,416	108	7.63	---	---	---
1941	1,751	134	7.65	8,587,344	603,325	7.03
1942	1,693	205	12.11	8,987,159	881,420	9.81
1943	2,569	151	5.88	14,549,397	703,982	4.84
1944	2,926	259	8.85	17,341,932	1,362,070	7.85
1945	3,229	197	6.10	21,162,392	1,114,849	5.27
1946	3,226	247	7.66	22,027,661	1,334,078	6.06